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GB 1484033
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(58) Field of search
G2J
G1M

(54) Grating film for quantitative or semi-quantitative microscopic evaluation of biological specimens

(57) A transparent film for use in making measurements under a light microscope, which film has reproduced photographically thereon a grid. The film may be used as a slide cover.

The drawings originally filed were informal and the print here reproduced is taken from a later filed formal copy.

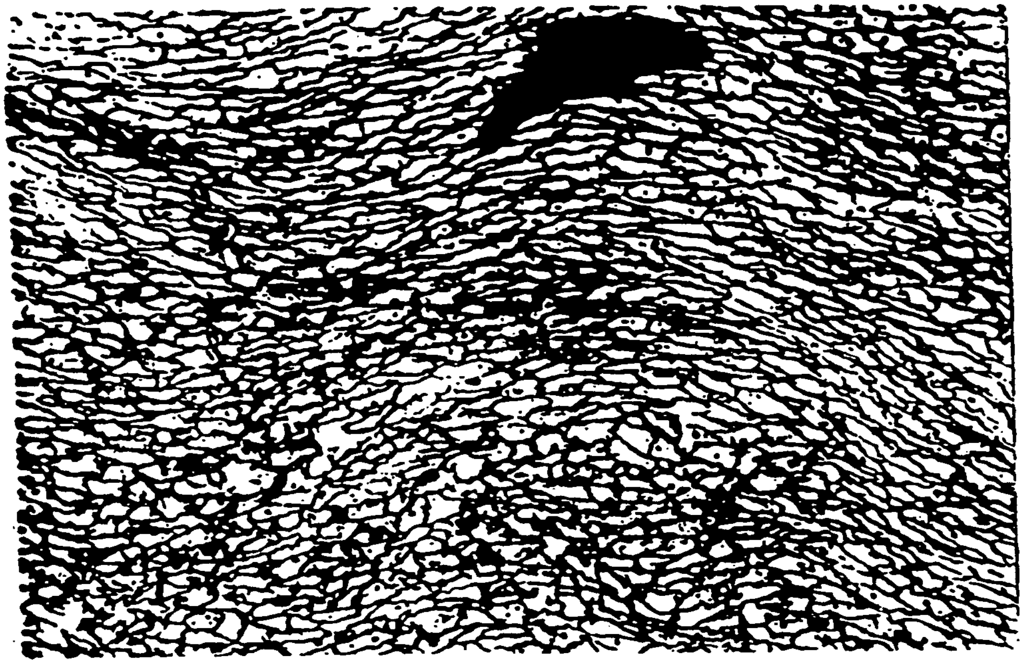

Fig.1.

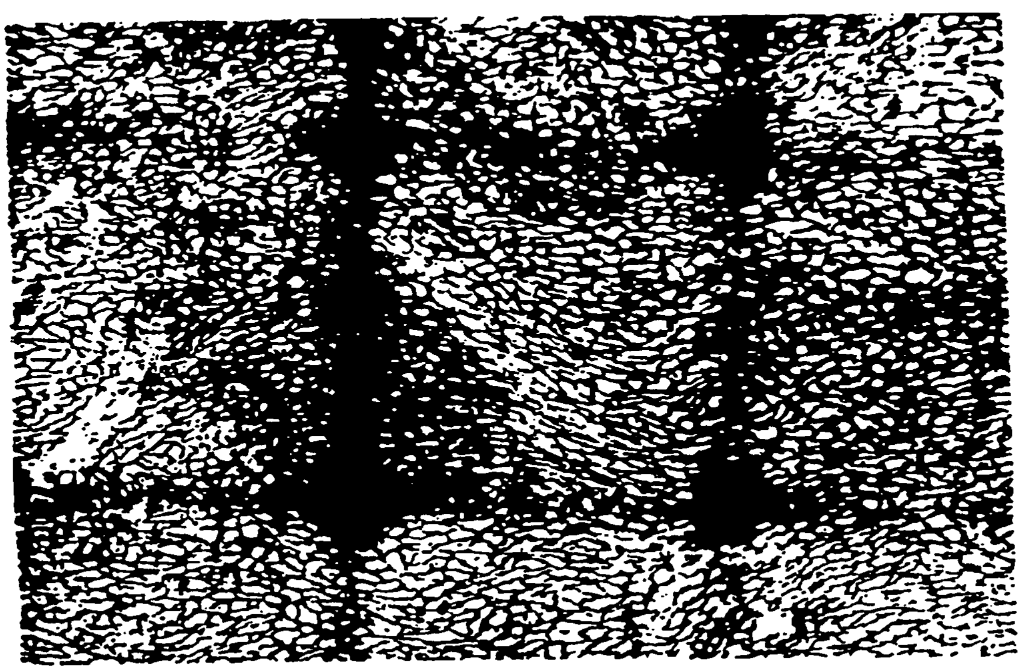

Fig.2.

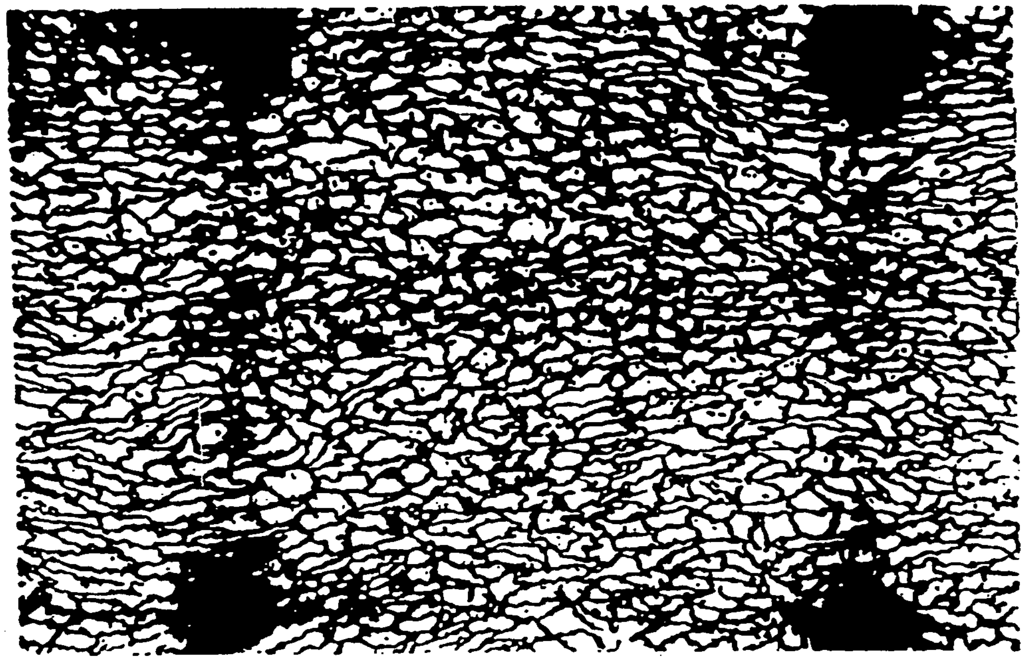

Fig.3.

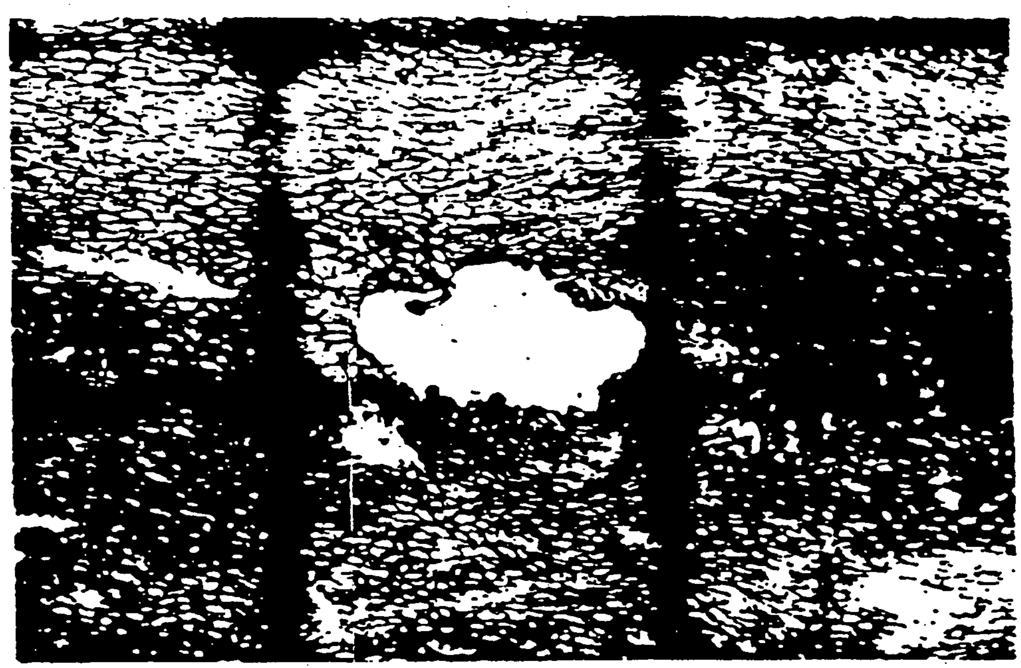

Fig.4.

SPECIFICATION

Grating film for quantitative or semi-quantitative microscopic evaluation of biological specimens

The present invention relates to the measurement under a light microscope of a specimen such as a biological specimen.

In biological research it is becoming increasingly important to have instruments that provide reproducible and precise quantitative or numerical results. In this specific field the market offers nothing meeting these requirements for quantitative analysis of biological specimens.

At present it is practically impossible during light microscopy examination of specimens to locate and map a specific area. This is particularly so if the area is larger than one field in the objective (e.g. examining the thoracic aorta of rats, with a surface of about 2 cm^2). It is thus difficult to state the exact area of a specimen or, if an alteration is present, to assess the abnormal surface as a percentage of the normal and, again, to map the distribution of the affected area.

The market currently offers grid-marked eye-pieces which can be mounted on a microscope. Such an eye-piece enables the observer to measure area under a single optical field but not to compare it with the rest of the specimen under examination because the grid remains fixed and cannot be shifted with the whole specimen. Consequently, it is impossible to make a precise analysis of the whole of a specimen's surface without running the risk of skipping some areas or reading some parts of the surface more than once.

There are various ways of overcoming these difficulties. The areas already examined can be marked on the cover-slide by hand. The specimen can be shifted in relation to movements of the transfer-table made by turning the knob, etc. However, these procedures are imprecise and time-consuming.

In order to overcome these drawbacks, the present invention provides a transparent film, suitable for use in making measurements under a light microscope, which film has reproduced photographically thereon a grid. Preferably, the grid has been derived by photographically reducing in size a larger sized grid.

The present invention also provides a process for preparing such a film, which process comprises photographically reproducing on a transparent film a grid. Preferably, a larger sized original of the grid is photographically reduced in size and reproduced photographically on the film.

The grid on the film may be made up of any desired pattern of lines. For example, the grid may consist of a series of regularly spaced apart parallel lines or concentric circles. Preferably, the grid consists of identically sized squares (a grating film), or rectangles.

When a larger original of the grid is reduced photographically in size, the desired size of the grid on the transparent film will determine by how large a factor the original is reduced in size. The grid must be small enough to enable a part of it to be seen when viewed through a light microscope at an appropriate magnification. When the grid consists of squares, for example, the desired size of the squares on the film will determine how greatly an original of a grid needs to be reduced in size. Typically, however, there may be a reduction in dimensions such as the length of a side of a square by a factor of 10 or more, preferably of from 10 to 50. A grid of squares each side of which has a length of 1.0 mm or less, for example of 0.1, 0.25, 0.5 or 1.0 mm, can thus be produced. A series of parallel lines or concentric circles may be spaced apart by these distances.

Any suitable photographic film onto which the grid may be reproduced can be employed. Kodalith ortho 9 × 12 cm Type 3 photographic film plates with 0.1mm Estar support may be used. The plates can be cut to the desired size and shape using ordinary scissors, which makes the invention highly adaptable. Normally, a rectangle of film according to the invention 1.5 by 3.5 cm or 1.5 by 5.5 cm can be used to cover a microscopic slide. The film should have minimal thickness so that it does not interfere with the optics of the microscope.

The film of the invention can be used for quantitative or semi-quantitative evaluation of specimens, for example biological specimens, under a light microscope without compromising qualitative assessment. Biological specimens for microscopic examination, of whatever type, can be observed adhering to or lying on a solid transparent (glass) stand. Examples are cell monolayers from tissue culture, thin and ultra-thin tissue sections, cell cultures, etc. Observation of such specimens by means of the present invention enables the area examined to be determined numerically (e.g. number of cells/mm^2 present on the surface of a fixed area of tissue of cell culture specimens), or the thickness of a vessel wall to be assessed quantitatively.

Accordingly, the invention further provides a method for carrying out a measurement on a specimen, for example a biological specimen, under a light microscope, which method comprises positioning a transparent film according to the invention over a specimen carried on a solid transparent support and carrying out the desired measurement relating to the specimen under the microscope.

After having been used for specimen analysis a film according to the invention can be washed with distilled water and absolute ethanol and gently dried with filter paper, then re-used. During the drying step some care must be taken not to damage the photographic emulsion by rubbing.

The grating film can thus be re-used several times or, if needed, can be left on the biological specimens as a cover-slide to store specimens for long periods of time. Long-term storage of specimens is

desirable or required, for example, in toxicity studies where the proof of the safety of a compound must be kept for years.

The invention therefore additionally provides a method of mounting a specimen, such as a biological specimen, for use in light microscopy, which method comprises mounting a specimen on a microscope slide, employing a transparent film according to the invention as a cover slide.

If specimens are assembled in aqueous substances or in carriers which can solidify, the cover-slides must be attached to the slide with an adhesive in order to prevent the specimens drying. The film can seal the specimen in place either with aqueous carriers (e.g. Kaiser's glycerinated gelatine) or with natural resins (e.g. balsam of Canada) or synthetic resin.

In the Figures of the accompanying drawings:

Figure 1 is a photograph of an endothelial cell monolayer from the thoracic aorta of a healthy rat as it appear under a light microscope using a common cover slide;

Figure 2 is a photograph of the same specimen as in Figure 1 with a film according to the invention laid over it, the squares of the grid being 0.5 mm by 0.5 mm;

Figure 3 is the same as Figure 2 but with a greater magnification; and

Figure 4 is a photograph showing a hole (center of Figure) which is the opening of a small artery branching off the thoracic aorta to the periphery (intercostal artery), the squares of the grid being 0.5 mm by 0.5 mm.

The grid in Figure 4 can be used to measure the size of the opening of the small artery. The lengths of the major and minor axes of the opening are 0.37 mm and 0.2 mm respectively.

The following Examples illustrate the invention.

Example 1

A grid of squares 0.5 cm/side, each with an area of 0.25 cm² was drawn on transparent paper using black Indian ink. The grid was then photographically reduced to squares 0.5 mm/side and at the end the grating was photographically reproduced in a 1/1 ratio on Kodalith ortho 9 × 12 cm Type 3 photographic film plates with 0.1 mm Estar support.

Example 2

Figure 3 of the accompanying drawings shows a single square of a 0.25 mm² square grid film according to the invention. Using the film, the endothelial layer of rat thoracic aortas was analysed in order to detect injuries to endothelial cells after treatment with a high-cholesterol diet. The integrity of the endothelial layer was assessed observing the presence of endothelial reticulum previously stained with silver nitrate. Injured areas were characterized by the absence of endothelial reticulum but presence of the nuclei or/and the presence of smooth muscle cells.

Each 0.25 mm² square was divided into four quarters (0.0625 mm² each) and a evaluation was made for each quarter observing the presence of endothelial reticulum, smooth muscle cells and endothelial nuclei.

This gave a surface divided as follows:

- complete endothelial reticulum	106.5 mm²
- endothelial nuclei	18.812 mm²
- smooth muscle cells	5.437 mm²
- total surface checked	130.749 mm²

of which 14.39% with mild injury (nuclei) and 4.16% with severe injury (smooth muscle cells).

It is thus clear that the invention permits full numerical analysis of the specimen under examination.

Example 3

A film according to the invention was employed in the evaluation of cellular colonies in culture (e.g. fibroblasts, aortic smooth muscle cells, endothelial cells). The invention enabled the number of cells per colony to be counted, to determine the size of the colonies in mm², and to calculate the rate of growth of colonies by checking the culture at successive times.

Smooth muscle cell cultures were grown from thoracic aortas of rabbits with two different sera (CS=Control Serum; CS+D=Control Serum+Drug) in order to check if the drug could alter the normal cellular growth.

These cultures were maintained in 24-well cell culture Cluster Dishes supplied by M.A.Bioproducts (1.6 cm of diameter for each well) under sterile conditions.

Dishes of film, according to the invention, were cut with a diameter of 1.5 cm in order to put them into the wells where smooth muscle cell cultures were grown and light microscope analyses carried out.

The dishes of grating film, according to the invention, were placed directly over the growing cultures. A grid of 0.5 mm/side was used when the growth of the cell colonies was in the beginning, and a grid of 1 mm/side was preferred when the cell colonies had larger size.

The growth rate of living cultures was followed by determination of their area after different times of incubation until the stationary growth phase was reached (5 weeks).

The area of each colony in culture was calculated in mm^2.

Time of incubation	Mean area of smooth muscle cell colonies checked by light microscope / mm^2 CS*	CS+D[1]	Percentage of cellular inhibition of drug
1 week	1.85	1.65	10.81
2 weeks	2.45	2.18	11.02
3 weeks	23.78	21.30	10.42
4 weeks	74.61	51.41	31.09
5 weeks	131.87	75.10	43.04
rate of growth (after 5 weeks)	3.8 mm^2/day	2.1 mm^2/day	

*CS = Control Serum
1)CS+D = Control Serum + Drug.

From the data obtained checking the colony areas we could calculate both the rate of growth of colonies (in this example 3.8 mm^2/day for smooth muscle cell cultures maintained in Control Serum and 2.1 mm^2/day for those in Control Serum+Drug after 5 weeks of incubation), and an eventual effect on the cellular growth induced by the drug treatment (in this case we had at the end of the treatment an inhibition of about 43%).

This approach offers so the following advantages:

1) reduced handling of cultures because they can be analysed *in loco*;
2) no treatment of cells with lytic enzymes.

During cell digestion with trypsin and during centrifugation some cells are normally lost;

3) As a consequence of points 1 and 2, greater precision and considerable savings in working time are achieved.

CLAIMS

1. A transparent film suitable for use in making measurements under a light microscope, which film has reproduced photographically thereon a grid.
2. A film according to claim 1, wherein the grid has been derived by photographically reducing in size a larger grid.
3. A film according to claim 2, wherein there has been a reduction in dimensions by a factor of from 10 to 50.
4. A film according to any one of the preceding claims, wherein the grid on the film consists of identically sized squares.
5. A film according to claim 4, wherein a side of the said squares has a length of 0.1, 0.25, 0.5 or 1.0 mm.
6. A process for the preparation of a transparent film as claimed in any one of the preceding claims, which process comprises reproducing photographically on a transparent film a grid.
7. A process according to claim 6, wherein a larger original of the grid is photographically reduced in size and reproduced photographically on the film.
8. A transparent film, suitable for use in making measurements under a light microscope, substantially as hereinbefore described.
9. A transparent film suitable for use in making measurements under a light microscope, substantially as hereinbefore described in Example 1.
10. A process for the preparation of a transparent film, suitable for use in making measurements under a light microscope, said process being substantially as hereinbefore described.
11. A process for the preparation of a transparent film suitable for use in making measurements under a light microscope, said process being substantially as hereinbefore described in Example 1.
12. A method for carrying out a measurement on a specimen under a light microscope, which method comprises positioning a transparent film as claimed in any one of claims 1 to 5, 8 or 9 or which has been produced by a process as claimed in any one of claims 6, 7, 10 or 11 over a specimen carried on a solid transparent support and carrying out the desired measurement relating to the specimen under the microscope.
13. A method according to claim 12, wherein the specimen is a biological specimen.
14. A method for carrying out a measurement on a specimen under a light microscope, said method being substantially as hereinbefore described in Example 2 or 3.
15. A method of mounting a specimen for use in light microscopy, which method comprises mounting a specimen on a microscope slide, employing a transparent film as claimed in any one of claims 1 to

5, 8 or 9 or which has been produced by a process as claimed in any one of claims 6, 7, 10 or 11 as a cover slide.

16. A method according to claim 15, wherein the specimen is a biological specimen.

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